Bacterial heterotrophy in AOM enrichments

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Consortia of anaerobic methanotrophic archaea (ANME) and sulfate-reducing bacteria are responsible for the anaerobic oxidation of methane (AOM) in marine sediments. Laboratory enrichments of such consortia have been frequently obtained but were never devoid of community members not involved in AOM, even after years of repeated dilution and inoculation. In order to understand their metabolism and potential interaction with consortia members, we used a lipid stable isotope probing approach and performed incubations of meso- and thermophilic ANME-1 dominated enrichments from the Guaymas Basin with L-3-^{13}C-Leucine (3-Leu) as organic carbon source for potential heterotrophic activity. Most of the ^{13}C from 3-Leu was incorporated into bacterial fatty acids, especially iso and anteiso-branched $C_{15:0}$ and $C_{17:0}$ as well as monounsaturated $C_{18:1\omega9}$ and $C_{18:1\omega7}$. We observed no direct assimilation of 3-Leu into archaeal tetraethers. These incorporation patterns were independent of whether CH_4 was added to the enrichments or not. Direct tests with the partner HotSeep-1 pure culture isolated from the thermophilic AOM enrichment did not show any ^{13}C-incorporation. In combination, our observations suggest that 3-Leu was utilized only by heterotrophic bacteria but not by AOM consortia members. Moreover, this indicates that iso and anteiso-branched $C_{15:0}$ and $C_{17:0}$ and monounsaturated C_{18} fatty acids are predominantly derived from bacterial heterotrophs that likely thrive on ^{13}C-depleted necromass of AOM-performing microorganism.